III. Specification Amendments

(¶ on page 9, line 9)

The level of protein homology can be determined with the computer program "BLAST 2 SEQUENCES" by selecting sub-program: :BLASTP", that can be found at www.ncbi.nlm.gov/blast/bl2seq/bl2.html ~~www.ncbi.nlm.gov/blast/bl2seq/bl2.html~~.

(¶ on page 13, line 18)

Vaccines according to the present invention may in a preferred presentation also contain an adjuvant. Adjuvants in general comprise substances that boost the immune response of the host in a non-specific manner. A number of different adjuvants are known in the art. Examples of adjuvants are Freunds Complete and Incomplete adjuvant, vitamin E, non-ionic block polymers, muramyldipeptides, saponins such as QUILL A ~~Quill A~~(R), mineral oil e.g. BAYOL ~~Bayol~~(R) or MARKOL ~~Markol~~(R), vegetable oil, and CARBOPOL ~~Carbopol~~(R) (a homopolymer), or DILUVAC ~~Diluvac~~(R) Forte.

(¶ on page 19, line 32)

In order to obtain the full-length *BCVIR* cDNA sequence, the missing 5' end was obtained as follows: the cDNA library was used as a DNA template for a PCR experiment using the T3 sense universal primer derived from the vector sequence (located 70 bp upstream of the *Eco*RI cloning site) and an antisense primer P2 (5'-ATGAGTCTATTGACTCCTTG-3'(SEQ. ID. NO.: 15)) derived from the *BCVIR* cDNA sequence (nucleotide position 476-496 downstream of the *Eco*RI cloning site in the complete sequence of the cDNA, see Fig. 1 and Fig.2). The PCR was performed as described below (§ PCR amplification) using 1 µl of the cDNA library (10^5

phages) as DNA template. The highest resulting PCR fragment (with a size of around 550 bp) was gel-extracted using gel extraction spin columns (Genomed), cloned in pGEM®-T vector using the pGEM®-T vector System II kit under the recommendations of Promega and sequenced on both strands as described behind, using T7 and SP6 universal primers (Genome Express).

(¶ on page 20, line 13)

Amplifications were performed in a PTC-100™ Programmable Thermal Controller (MJ Research, INC.) as described in Carret *et al.* (1999, *J. Eukaryot. Microbiol.*, **46(3)**, 298-303) with the following conditions : 3 min. at 94°C, 30 cycles of 1 min. at 94°C, 1 min. at 55°C and 1 min. at 72°C, and then 5 min. at 72°C. Primers P1 (sense 5'-GACGTTTGATGTGATGAGGGAAGC-3'(SEQ. ID. NO.: 12)), P5 (sense 5'-AGGGAGCTGTCACGGAAGATT-3'(SEQ. ID. NO.: 13)), P2 (antisense) and P15.2 (antisense 5'-AATGACATACTCACAGGAAGC-3'(SEQ. ID. NO.: 14)) were all derived from the *BCVIR* cDNA sequence (their respective position is indicated on the Fig.1) and commonly used for the molecular analysis of this sequence.

(¶ on page 21, line 11)

RT-PCR. RT-PCR was performed using the two-step protocol of the Enhanced Avian RT-PCR kit as described by the manufacturer (Sigma). Briefly, the first step (retrotranscription) was performed for 50 min. at 42°C using 250 pg of total RNA as template and 1 μM of specific cDNA primer (sense P1, sense P5 (5'-AGGGAGCTGTCACGGAAGATT-3'(SEQ. ID. NO.: 13)) or antisense P15.2), and the second step was performed at an annealing temperature of 50°C as described above (§PCR amplification), using one fifth of the first step reaction as a template.

As controls, the first step was also performed without eAMV-RT for each primer tested for DNA contamination, and the experiments were also performed with specific primers (sense Bcc12D3.1 and antisense Bcc12D3.2) derived from the Bcc12D3 cDNA of *B. canis* A.

(¶ on page 21, line 23)

Northern Blotting. For Northern blot experiments, total RNA was electrophoresed through gel containing formaldehyde and transferred to Nylon membrane as described in Maniatis/Sambrook (Sambrook J. et al., Molecular cloning: a laboratory manual. ISBN 0-87969-309-6). Hybridisation was performed with a probe derived from a P1/P2 PCR fragment amplified on the *BCVIR* cDNA sequence and labelled with digoxigenin (DIG)-dUTP according the DIG High Prime DNA labelling kit (Boehringer Mannheim). Total RNA samples depleted in mRNA were also used for hybridisation experiments and mRNA depletion was performed using POLYA TRACT ISOLATION SYSTEMS ~~PolyA Tract mRNA Isolation Systems~~® III kit (Promega).

(¶ on page 37, line 32)

***In Vitro* translation of the *BCVIR* cDNA sequence.** The ability of the *BCVIR* cDNA to direct frame shifting was examined in an *in vitro*-translation system. *In vitro*-translation products were synthesised using the TNT® Quick Coupled Transcription/Translation System (Promega) with a PCR DNA fragment containing the entire *BCVIR* cDNA sequence, a Kozacks sequence and a T7 promoting sequence at its 5' end for the *in vitro* translation. In order to amplify such a DNA fragment, the PCR was performed on the pBK-CMV plasmid carrying the incomplete 1041 bp *BCVIR* cDNA as DNA template using the two following primers: a 90 mer sense oligonucleotide (primer P0 : 5'-

GGATCCTAATACGACTCACTATAGGGAGACCACCATGGAGTCGACATCAACAG ACCAACTTTGTTGCCGAGAACCGTCCCACCTTTGG-3'(SEQ. ID. NO.: 14)), containing a Kozacks and a T7 promoting sequences but also the missing 5' end of the entire *BCVIR* cDNA sequence containing the starting ATG codon of the ORF1 (bolded in the primer sequence; nucleotide position 75-130 on the complete cDNA sequence; see Fig.1 and Fig.2), and the antisense primer P15.2 from the *BCVIR* cDNA sequence. The PCR was performed using 100 ng of the circular recombinant pBK-CMV plasmid and the following conditions of amplification were used: 1 cycle of 3 min. at 94°C; 5 cycles of 1 min. at 94°C, 1 min. at 37°C and 1 min. at 72°C; 25 cycles of 1 min. at 94°C, 1 min. at 50°C and 1 min. at 72°C. The PCR products were gel extracted twice to ensure their purity and resuspended in nuclease free water at a concentration of 100 ng/µl. Then, they were added into a reticulocyte lysate to be translated using the TNT® Quick Coupled Transcription/Translation System as described by the manufacturer (Promega). As control, the same experiment was performed by omitting the PCR fragment in the reaction. The total radiolabeled translated products (5 µl of the reaction) and immunoprecipitated products (from 20 µl of the reaction) with the anti-GST-ORF1 or the anti-GST-ORF2 or the anti-GST antisera and the pre-immune rabbit serum were separated on a 15% SDS-PAGE.